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CLAIMS

1. A method for the objective determination of the total concentration of sperm cells in a semen sample and the proportion of live sperm cells therein, comprising subjecting the semen sample or a diluted subsample of the semen sample to selective staining and determining the total concentration of the sperm cells and the proportion of live sperm cells by means of a detection means responsive to the selective staining, wherein the determination of the total concentration of sperm cells and of the proportion of live sperm cells are performed using the same sample or subsample and in the same determination routine.

2. A method according to claim 1, wherein the determination of the total concentration of sperm cells and of the proportion of live sperm cells are performed substantially simultaneously.

3. A method according to claim 2, wherein the determination of the total concentration of sperm cells and of the proportion of live sperm cells are performed in the same determination operation.

4. A method according to any of claims 1-3, wherein the selective staining comprises a staining which stains all sperm cells combined with a staining which selectively stains dead cells.

5. A method according to any of the preceding claims, wherein any dilution of the sample has been performed using a diluent which sustains viability of the sperm cells during the determination.

6. A method according to any of the preceding claims, wherein the selective staining is performed using one or more fluorochromes resulting in fluorescent qualities being conferred to live sperm cells and dead sperm cells, the fluorescent quality or qualities of live cells being distinguishable, by the detection means, from the fluorescent quality or qualities of dead sperm cells, and the determination is performed by selective counting of cells of each fluorescent quality.

7. A method according to any of the preceding claims, wherein the proportion of dying sperm cells is also determined, the selective staining being adapted to allow distinction, by the detection means, between dying sperm cells and on the one hand dead sperm cells and on the other hand live sperm cells.

8. A method according to claim 7, wherein the selective staining is performed using one or more fluorochromes resulting in fluorescent qualities being conferred to live sperm cells, dead sperm cells and dying sperm cells, the fluorescent quality or qualities of live sperm cells, dead sperm cells and dying sperm cells being distinguishable from each other by the detection means, and the determination is performed by selective counting of cells of each fluorescent quality.

9. A method according to any of claims 6-8, wherein the fluorochromes are fluorochromes binding to DNA.

10. A method according to claim 9, wherein the fluorochromes comprise a fluorochrome capable of selectively staining dead or dying sperm cells, this fluorochrome being capable of entering a sperm cell through a leaking or defect plasma membrane, but substantially incapable of entering a sperm cell having an intact plasma membrane, and another fluorochrome capable of staining all sperm cells, this fluorochrome being capable of entering a cell through an intact cell membrane.

11. A method according to any of claims 6-10, wherein the excitation of the fluorochromes is performed by means of light in the wavelength range about 488 nm, the fluorochrome staining all sperm cells being SYBR-14, and the fluorochrome staining the dead or dying sperm cells being propidium iodide.

12. A method according to any of claims 6-10, wherein the excitation of the fluorochromes is performed by means of light in the wavelength range about 543 nm, the fluorochrome staining all sperm cells being MPR71292, and the fluorochrome staining the dead or dying cells being ethidium-homodimer-2, EHD2.

13. A method according to any of claims 6-12, wherein the fluorochrome staining all sperm cells is used in total concentrations below standard total concentrations conventionally applied for such fluorochromes.

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14. A method according to any of claims 6-13, wherein the fluorochrome staining all sperm cells is used in total concentrations in the range from 25 to 75 nanomolar.

15. A method according to claim 14, wherein the fluorochrome staining all sperm cells is used in total concentrations about 50 nanomolar.

16. A method according to any of claims 6-15, wherein the staining of the sperm cells is performed at a temperature below 35°C.

17. A method according to claim 16, wherein the staining of the sperm cells is performed at a temperature of at the most 30°C.

18. A method according to claim 17, wherein the staining of the sperm cells is performed at a temperature between 15°C and 25°C.

19. A method according to claim 18, wherein the staining of the cells is performed at room temperature.

20. A method according to any of the preceding claims, wherein the sample or subsample is combined with an internal concentration standard means, and the determination of the total concentration of the sperm cells and the proportion of live sperm cells are performed simultaneously by means of a detection means responsive to the selective staining and to the internal concentration standard means.

21. A method according to claim 20, wherein the internal concentration standard means is constituted by standardisation particles, the standardisation particles being added in a predetermined number per weight or volume amount of the sample or subsample.

22. A method according to claim 20 or 21, wherein the standardisation particles are fluorescent particles having a fluorescent quality distinguishable from the fluorescent qualities of the live sperm cells, dead sperm cells, and dying sperm cells.

23. A method according to any of claims 20-22, wherein the detection means comprises a flow cytometer.

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24. A method according to any of claims 20-22, wherein the detection m ans comprises a laser scanning cytometer.

25. A method according to any of claims 20-24, wherein the size and total sperm cell concentration of a subsample are adapted so that the number of sperm cells corresponds to between one tenth and ten times the number of standardisation particles.

26. A method according to claim 25, wherein the size and total sperm cell concentration of the subsample are adapted so that the number of sperm cells corresponds to between one quarter and four times the number of standardisation particles.

27. A method according to claim 26, wherein the size and total sperm cell concentration of the subsample are adapted so that the number of sperm cells corresponds to between half and twice the number of standardisation particles.

28. A method according to any of claims 20-27, wherein the diluent is a diluent containing protein.

29. A method according to claim 28, wherein the protein is BSA.

30. A method according to any of claims 20-27, wherein the diluent is a diluent containing polyvinyl alcohol.

31. A method according to any of the preceding claims, wherein the determination of the total concentration of the sperm cells and the proportion of live sperm cells are determined as a mean value of the determination of the total concentration of the sperm cells and the proportion of live sperm cells performed on two or more subsamples of a semen sample.

32. A method for predicting the likelihood of fertilizing a female animal by artificial insemination with an insemination dose, comprising determining the total concentration of sperm cells in the semen sample from which the insemination dose is taken or is to be taken, and the proportion of live sperm cells therein by a method according to any of claims 1-31, and including the thus determined total concentration of the sperm cells in the semen sample and the proportion of live sperm cells therein, or the concentration,

calculable therefrom, of live sperm cells in the sample, in the parameters on the basis of which the likelihood of fertilizing the animal is predicted.

33. A method according to claim 32, wherein the likelihood of fertilizing the female animal is predicted on the basis of the determined total concentration of the sperm cells in the semen sample and the proportion of live sperm cells therein, or the concentration, calculable therefrom, of live sperm cells in the sample.

34. A method according to claim 32 or 33, wherein the prediction of the likelihood of fertilizing the female animal is performed on the basis of statistically significant correlations between fertility data obtained in insemination experiments with several female animals and data indicating the total concentration of the sperm cells in the semen sample used in the insemination experiments and the proportion of live sperm cells therein, and/or data indicating the concentration of live sperm cells therein.

35. A method according to any of claims 32-34, wherein the female animal is a multiparous animal, and the number of offspring resulting from the fertilization is also predicted.

36. A method according to any of claims 32-35, wherein the semen sample is a fresh ejaculate.

37. A method according to any of claims 32-35, wherein the semen sample is a frozen insemination dose, the sample being thawed before being subjected to the determination method.

38. A method according to claim 37, wherein data obtained by the determination method performed on the fresh ejaculate from which the insemination dose was taken are included together with data obtained by the determination method performed on the insemination dose.

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39. A method for artificial insemintion of a female animal, comprising predicting th likelihood of fertilizing a female animal, the prediction comprising

determining the total concentration of sperm cells in the semen sample from which the insemination dose is taken or is to be taken, and the proportion of live sperm cells therein by a method according to any of claims 1-32, and including the thus determined total concentration of the sperm cells in the semen sample and the proportion of live sperm cells therein, or the concentration, calculable therefrom, of live sperm cells in the sample, in the parameters on the basis of which the likelilhood of fertilizing the animal is predicted, and

on basis of the predicted likelihood selecting an insemination dose for use with artificial insemination of the female animal.

40. A method according to claim 39, wherein the female animal is a multiparous animal, and the insemination dose is an insemination dose having a predicted likelihood of resulting in a number of offspring above a predetermined discrimination number.

41. A method according to claim 39 or 40, wherein the likelihood of fertilizing the female animal is predicted on the basis of the determined total concentration of the sperm cells in the semen sample and the proportion of live sperm cells therein, or the concentration, calculable therefrom, of live sperm cells in the sample.

42. A method according to any of claims 39-41, wherein the prediction of the likelihood of fertilizing the female animal is performed on the basis of statistically significant correlations between fertility data obtained in insemination experiments with several female animals and data indicating the total concentration of the sperm cells in the semen sample used in the insemination experiments and the proportion of live sperm cells therein, and/or data indicating the concentration of live sperm cells therein.

43. A method according to any of claims 39-42, wherein the semen sample is a fresh ejaculate.

44. A method according to any of claims 39-42, wherein the semen sample is a frozen insemination dose, the sample being thawed before being subjected to the determination method.